An Experimental Approach to Evaluate the Effects of Low Dissolved Oxygen Acting Singly and in Binary Combination with Toxicants on Larval Atlantic Sturgeon, *Acipenser oxyrinchus oxyrinchus*

Final Report (revised)

This Final Report covers activities conducted by awardees at NYU School of Medicine and the NOAA Northeast Fisheries Science Center Howard Laboratory initiated in April 2015, continuing with experimental assessment of effects of contaminants and dissolved oxygen on Atlantic sturgeon larvae from July through November 2015, and follow-up analyses of those data.

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INTRODUCTION

All populations of Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) south of the Gulf of Maine are listed as "endangered" under the U.S. Endangered Species Act including the Hudson River and Delaware River populations in the New York Bight Distinct Population Segment (DPS). In the 1890s, the Delaware River supported the largest fishery for Atlantic sturgeon coast wide (Secor and Waldman 1999) with adult female abundance estimated at 179,000 individuals versus 54,000 individuals from all other U.S. populations of sturgeon combined. Since that time, overharvest of spawning adult Atlantic sturgeon within natal rivers, coastal harvest of adults and subadults, vessel strikes, and environmental degradation of sturgeon habitat in the Delaware River have reduced the population to a small fraction of historic levels. Although federally listed as five DPS in 2012, several Atlantic sturgeon populations appear to either have stabilized (e.g., Altamaha River, Georgia) or even increased in abundance, e.g., Hudson River, New York (Schueller and Peterson 2011, NYS DEC 2013). Despite that fact that the Hudson and Delaware River populations of Atlantic sturgeon are included in the same New York Bight DPS, the production of the two sturgeon nurseries appears to be on different trajectories. For example, abundances of juvenile Atlantic sturgeon in the Hudson River during fall of 2012 were the highest in recent record. The Delaware River population, however, has not rebounded and is reported to contain less than 300 spawning adults annually (Breece et al. 2013) with year-class production either very low or below the level of detection in most years. Over the past 50 yr, intensive efforts to collect young-of-the-year Atlantic sturgeon in the Delaware were unsuccessful until 2009, 2011, and 2014 although use recently of acoustic telemetry revealed small numbers of adult fish entering the system at spawning time. These observations suggest that environmental conditions in the Delaware River do not support recruitment in most years.

In addition to overharvest, environmental conditions have degraded overall in the Delaware River and doubtlessly contributed to recruitment failure of Atlantic sturgeon, but the environmental agents restricting recruitment have yet to be established. We hypothesize that low dissolved oxygen (DO) concentrations, operating singly or in combination with other environmental stressors such as chemical contaminants, (e.g., polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs)) have limited recruitment of Atlantic sturgeon in the Delaware River. Low DO has been a chronic problem for recruitment of other anadromous fish stocks in the Delaware and it is believed that the oxygen block reported in the 1950s and 1960s near Philadelphia almost extirpated the once-abundant American shad (*Alosa sapidissima*) and striped bass (*Morone saxitilis*) populations. DO levels have since increased due in part to the legislatively mandated target levels of 3.5 mg O_2/L DO set by the Delaware River Basin Commission (DRBC). This DO-minimum standard appears to have succeeded by contributing to the 1000-fold increase in size of the striped bass population and the stabilization of American shad abundances (Weisburg et al. 1996).

The Atlantic sturgeon population of the Delaware River, however, remains severely depressed suggesting that it may be more sensitive than other anadromous fishes in this system to low DO and other environmental stressors likely encountered in there and elsewhere. Sturgeons are noted for being hypersensitive to hypoxia and particularly so at higher temperatures (Klyashtorin 1982). These environmental conditions are likely to be

experienced by Atlantic sturgeon in early summer in the Delaware River which is coincidental with spawning and the presence of the sensitive early life-stages (ELS) (Chambers et al. 2012). Importantly, the target level of DO for the Delaware River of 3.5 mg O_2/L DO represents a 24-hr average. More extreme depressions of DO, possibly leading to severe hypoxia, are expected during the diel cycle with DO minima at night during periods of warm water. These hypoxic events are likely to overlap temporally (mid to late June) and spatially (e.g., Marcus Hook) with areas where Atlantic sturgeon are likely to spawn (Sharp 2010). Further, because sturgeon eggs are exceptionally large for a fish – especially one that spawns during summer – and with a massive allocation to yolk relative to that found in other fish taxa, sturgeon embryos require high DO concentrations in their immediate microenvironment. Sturgeon embryos also have a protracted embryonic period for a summer spawning fish. Further, the embryos and recently hatched larvae reside at the river bottom. Collectively, these life-history and habitat features of sturgeon place their ELS at especially high risks to hypoxia.

Fishes in the Delaware River are known to bioaccumulate high tissue burdens of persistent contaminants such as PCB, dioxins, and furans which are known to limit early-life success in many fishes (Tillitt et al. 2008). For example, Brundage (2002) reported that PCB and PCDD/Fs burdens in livers and ovaries of shortnose sturgeon (*Acipenser brevirostrum*) collected in the Delaware River were sufficiently high to potentially impede reproductive success. However, the sensitivities of fishes to PCBs and TCDD-induced toxicities are known to vary among species by several orders of magnitude (Elonen et al. 1998). Furthermore, in earlier controlled laboratory studies we reported that Atlantic sturgeon and shortnose sturgeon ELS were unusually sensitive to the toxic effects of a coplanar PCB (PCB126) and TCDD (Roy et al. 2011; Chambers et al. 2012). Importantly, these contaminants and low DO are likely to co-occur yet their interactive effects of these factors have yet to be investigated on ELS of any fish species. Despite the significant reduction in the release of these contaminants into the Delaware River system in recent years, exposure to sediment-borne contaminants such as PCBs and PCDD/Fs may be an increasing problem because of efforts to dredge portions of the estuary near Atlantic sturgeon nursery areas.

The critical DO habitat for Atlantic sturgeon ELS has yet to be empirically defined. However, it is known that ELS of fishes, including sturgeons, are far more sensitive to many stressors, including toxic chemicals such as PCBs and PCDDs, than juveniles and adults. It has been demonstrated that low DO limits the distribution of adult and juvenile Atlantic sturgeon in the Chesapeake and that juvenile and 1-yr old sturgeons are more sensitive to low DO than juveniles of other fish species, particularly at elevated temperatures and salinities (Niklitschek and Secor. 2009). To date, however, no study has empirically quantified the sensitivity of ELS of Atlantic sturgeon (nor any sturgeon species) to the damaging effects of low DO. Quantification of the DO requirements of ELS of Atlantic sturgeon, including the potential interactions between DO and other environmental stressors, is a precursor to defining essential sturgeon habitat and to establishing appropriate minimal conditions of habitat quality that would support enhanced Atlantic sturgeon ELS success and recruitment in the Delaware.

OBJECTIVES

The objectives of this study were to begin to fill the information void on effects of DO and

contaminants, acting singly and in combination, on the ELS of Atlantic sturgeon. Atlantic sturgeon embryos were acutely exposed to graded doses of a coplanar PCB congener (PCB126), TCDD, and an environmentally relevant Aroclor mixture. Subsequently, short-term (< 24-hr duration) experiments on larvae were implemented to evaluate the sublethal responses to low DO, and the potential for interactions of DO with other environmental stressors such as PCBs and TCDD. This work complements other ongoing studies sponsored by the Hudson River Foundation (HRF), and NOAA's Office of Response and Restoration (ORR), and the Northeast Fisheries Science Center (NEFSC) that we are conducting on the effects of PCBs and climate change (warming) singly and in combination on ELS success of Atlantic sturgeon and shortnose sturgeon. Results from those other studies will be shared with DRBC once completed.

The experiments were structured to address two working hypotheses:

1. The current DRBC mandate of a daily average DO of 3.5 mg/L places ELS of Atlantic sturgeon in the Delaware River at risk to hypoxia due to high temporal frequency excursions into DO levels below the target average.

2. Co-exposure to low DO and toxicants stressors, such as the PCB Aroclor mixture, a toxic PCB congener (PCB126), and 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD), increases the risks of Atlantic sturgeon ELS to toxicity from hypoxia.

Unlike prior work, in our broader efforts funded by HRF, NOAA ORR, NOAA NEFSC, and this DRBC-funded work on response by larvae to DO and toxicants, we have implemented and tested these hypotheses using laboratory experimental methodologies on ELS of Atlantic sturgeon. Here, the effects of DO concentrations were our primary focus and the toxicants, including locally relevant concentrations and types (PCB Aroclor mixtures), was a secondary focus as a and potentially interacting stressor. In the past experiments, we have successfully defined the sensitivity of ELS of Atlantic sturgeon and shortnose sturgeon to one coplanar PCB congener (PCB126) and TCDD. Our results demonstrated that both sturgeons are among the most sensitive of fishes investigated to PCBs as quantified by altered gene expression (Roy et al. 2011), multiple teratogenic and morphological metrics, ontogenetic progression, eye development, and survival (Chambers et al. 2012).

APPROACH

We employed an experimental laboratory approach to meet our objectives. The general protocol requires four steps. First, fertilized eggs of Atlantic sturgeon were obtained from hatchery production from several sources that differ in geographic origin and thus environmental conditions under which the respective populations can be expected to have evolved. These sources represent two geographically disparate sources: the Saint John River, New Brunswick, Canada, and the Altamaha River, Georgia, USA.

Second, embryos were subjected to a variety of experimental treatments representing environmental stressors. The stressors included 1) controlled, short-term exposures to one of several dioxin-like compounds in order to establish standard concentration levels as embryos and 2) over a dozen different constant thermal regimes and the effects on embryonic development evaluated (HRF-funded study).

Third, embryos and larvae were maintained under previously established husbandry protocols until the surviving larvae from different toxicant histories were subjected to short-term DO challenges. A battery of ELS biological responses was chosen for its sensitivity to the contaminants and/or DO. The ELS bioindicators included ones specific to the age window of exposure to low levels of DO (e.g., mortality, prey consumption rate, and activity of larvae).

Fourth, null hypotheses associated with imposed factors (toxicant, DO) were tested. Specifically, the tested compound hypothesis was that environmental conditions of contaminant and/or DO levels had no effect on the suite of biological responses measured on sturgeon larvae.

METHODS

Egg collection and husbandry. We obtained fertilized eggs of Atlantic sturgeon from commercial aquaculture and research operations that we have used previously. From the northern reaches of the geographic range of Atlantic sturgeon, we acquired recently fertilized eggs from an aquaculture operation (Acadian Sturgeon and Caviar, Inc.) on the Saint John River, New Brunswick (NB). Atlantic sturgeon from southern reaches of the species range were obtained from the U.S. Fish and Wildlife federal hatchery at Bears Bluff, South Carolina (SC) that has captive Atlantic sturgeon broodstock from the Altamaha River, Georgia. These two contrasting and distant sources allowed us to compare the response of offspring from two populations that experience significantly different environmental regimes in regards to ambient temperature and DO levels. We surmised that offspring of Atlantic sturgeon from the Saint John River would be more sensitive to low DO than those from the Altamaha River given their population exposure histories. Use of offspring from two separate populations ensured more diversity in our sources and was intended to provide more certainty in our results and their applicability to the Delaware River population.

Fertilized eggs were either transported by NOAA staff (NB source) or shipped overnight (SC source) to the NOAA Howard Laboratory in Sandy Hook, New Jersey. In both cases, eggs were received within 2 to 3-d post-fertilization. Upon arrival at the NOAA Laboratory, the ~ 7,500 to 10,000 eggs were thinned, live eggs separated from dead ones, and live eggs counted into lots of 30 and placed into glass culture dishes (15 x 11 x 4 cm) with 250 mL of 0.1 parts per thousand (ppt) salt water. Eggs and larvae were maintained at one of three constant temperatures (15, 19, 23 °C), until use for HRF-funded toxicant studies or at 15 °C until use in this (DO-exposure) study. For eggs included in the contaminant treatments, exposure were initiated within 24 hr of arrival at the lab (details below).

For context and completeness, we describe the array of experiments that we have conducted on the effects of environmental stressors on Atlantic sturgeon which represents our larger, multi-party funded efforts. A similar, concurrent effort has been made by us using shortnose sturgeon as well. Below, we provide more detail and report on the DRBC-funded work.

Experiments – One-factor designs: Toxicant effects. In our prior Hudson River Foundation and NOAA-funded projects, we exposed recently fertilized Atlantic sturgeon eggs for 24 hr to one of six concentrations of aqueous solutions of TCDD or PCB congener 126 at order of magnitude increments. Concentrations ranged from 0.001 to 100 ppb for TCDD and 0.01 to 1,000 ppb for

PCB126. The 24-hr exposure duration was selected because we had empirically determined in previous studies (sturgeon embryos exposed to radiolabeled PCB126) that longer exposures time did not increase whole embryo burdens of the contaminant (Roy et al. 2011).

In our earlier work (Chambers et al. 2012), and in order to meet the objectives of a 2015, HRF-funded single-factor toxicants experiment, we exposed Atlantic sturgeon eggs to one of seven different doses of TCDD, PCB126, or an environmentally relevant Aroclor mixture consisting of Aroclor 1248 (40%), Aroclor 1254 (40%), Aroclor 1260 (20%) (Roy et al. 2018) as well as solvent (acetone) and water controls in triplicate. Exposures were for 24-hr duration. Maintenance of embryos and larvae followed earlier protocols. Those high-treatment frequency exposure experiments defined the threshold concentrations of contaminants that compromise ELS success and bracket concentrations that do not result in impairment based on our measured biological endpoints. We selected the toxicant concentrations for the DRBC-funded crossed designs (e.g., contaminant x DO) from those early trials to screen viable doses to maintain larvae to more advanced ages. This resulted in our use of three concentrations of TCDD, PCB126, and the Aroclor mixture for the contaminant x DO study reported here (Table 1). As before, exposures were for 24-hr duration, and the husbandry of embryos and larvae followed earlier protocols.

Experimental design: Toxicant x dissolved oxygen effects. We subjected recently fertilized Atlantic sturgeon eggs to one of three contaminants (PCB126, TCDD, or Aroclor mixture for 24-hr exposure), maintained the embryos and hatched larvae under uniform thermal and daylight regimes, and exposed larvae to one of five DO regimes. Trials at various DO levels were conducted on larvae ~ 3 to 16 weeks post hatching.

Maintenance of sturgeon larvae prior to DO experiments. Atlantic sturgeon larvae from the NB and SC sources were hatched and transferred to 5-L tanks with 0.01-ppt static salt water (saltwater source was a mixture of deioinized water and Aquavitro Salinity Salt). Rearing water was aerated with temperatures maintained at 15 °C and a light:dark regime of 14:10 hr. At 5-7 days after hatching, sturgeon larvae were offered prey (*Artemia*) at 0.5/mL, increasing with age on an *ad libidum* basis. Debris was siphoned from tanks every day. Half the rearing container volume was replaced with fresh water weekly. Age of larvae run in trials from varied from 19 to 111 days post hatch (dph).

Experimental dissolved oxygen system. A DO recirculating system was designed specifically for this research need. It was composed of five independent closed recirculating systems, with each system consisting of a connected set of containers (head tank, testing tank, and sump), a DO controller linked to nitrogen (N_2) gas to purge oxygen from water to target values, and test arenas for sturgeon larvae. Each recirculating system contained a 50-L head tank, two 17-L rectangular boxes (hereafter, boxes) with dimensions 40 × 33 × 15 cm (LWH) filled to 12 L and plumbed with an inflow and an outflow, and a 30-L sump tank (Figures 1, 2). The boxes housed the test arenas in which individual sturgeon larvae were placed. A Luminescent Dissolved Oxygen (LDO) sensor was placed in the head tank along with a micro air diffuser. The sensor was connected to a Hach SC200 controller which in turn was connected to a solenoid valve that controlled flow rate of compressed N_2 tank (304 Ultra High Purity) into the head tank via the micro air diffuser.

Water from the head tank was gravity fed into each of the two the boxes of each recirculating system. Overflow water from each box accumulated in a sump tank and was returned to the head tank by a submersible pump. Return water passed through a 5-um in-line filter cartridge mounted prior to the head tank.

Each box houses up to 12 test arenas. The arenas were fabricated from 7.6-cm (3-in) diameter PVC pipe (drain grade). A 195-um mesh floor was held in place by compression via a 3-in coupler slid over the bottom of the pipe. The couplers, through which four, 2-cm holes had been drilled to promote water flow through each arena, served as an elevated base for the arena. Arena water depth (~ 6 cm) and volume (~290 mL) were standardized across all arenas in each box. All containers had lids and the boxes had a gasket-sealed glass lid to minimize absorption of room O_2 by the DO test water. The DO system was located in a 15 °C temperature control box with a 14:10 hr light:dark regime.

Experimental protocol. Larvae of various ages and sizes, and each from a specific toxicant exposure history, were subjected to one of five concentrations of DO during short-term (21-hr) trials (Table 1). The doses were nominally 10, 8, 6, 4, and 3 mg O_2/L, and the duration of the trial was intended to mimic a short-term (e.g., overnight) O_2 bottleneck as might occur *in situ* as larvae transport down river through zones off different degrees of hypoxia. Larvae were maintained in group-rearing conditions (described above) after hatching and prior to the trial, at which time all surviving larvae were used. Larvae died during the husbandry phase prior to the DO trials which resulting in not all toxicant exposure concentrations being represented in the DO trials.

Larvae were transferred to individual arenas in the DO experimental system and maintained at 15 °C and 0.01-ppt salinity for the entirety of the trial. Larvae were acclimated to the experimental arenas at normoxic conditions (>9.5 mg O_2/L) and food withheld prior to initiation of DO reduction to target levels (acclimation / starvation period increased with fish age and size from 1 to 24 hr). After acclimation, the DO levels were reduced by 1 mg O_2/L per hr until the target DO level was achieved. Once at target DO levels, prey were added to each arena and the trial clock was initiated. Prey used were instar II *Artemia* enriched with Easy DHA Selco (Inve Aquaculture), an aquatic oligochaete (white worms, *Enchytraeus albidus*), or thawed frozen bloodworms (*Chironomus* sp.). Each trial lasted 21 hr at target DO levels and covered periods of light (p.m.), darkness (overnight, 10 hr), and light (a.m.). Low-intensity diffuse light was used during periods of daylight and an infrared (IR) light was on throughout the trial in order for IR-sensitive cameras to record larval activity during darkness. Larval removal after 21 hr at a constant DO target level defined the termination of the trial. Each fish was photographed in lateral perspective immediately after the trial was terminated and images were later used to determine fish size (standard length).

Response variables. Responses used to evaluate effects of depressed oxygen (i.e., hypoxia), acting alone or in combination with toxicants, were survival during the trial, activity level of larvae, and prey consumption during a trial. All responses scored are likely to be related to the probability of future survival of the young sturgeon in natural environments such as the Delaware River. These responses were operationally defined as follows.

Sturgeon larval mortality. Mortality occurred during the 21-hr trials. The total dead of initial numbers used per treatment combination were converted to proportions and normalized via angular transformation prior to analyses.

Prey consumption. Prey consumption levels were determined by difference of the counts of number of prey offered and complete counts of the number of prey remaining after the 21-hr trial. Upon completion of the trial, sturgeon larvae were quickly removed from the arenas and the number of remaining prey were rinsed into a clear dish, placed on a light table, and enumerated. Due to the three prey types used among the trials, each consumption count was converted to proportion of prey consumed during the trial, and data were angular-transformed prior to analyses.

Sturgeon larval activity. Activity of sturgeon larvae was quantified by analysis of video taken during two, 30-min segments of the overall trial period. One period was in the late p.m. (while still light) and after the target DO level had been achieved. The second period was in the a.m., after lights on. Each video was recorded and simultaneously run through EthoVision tracking software. All videos were set up to track the movement using center point detection of each larva in real time and concurrently for up to 12 larvae in a box. An individual's average velocity (cm/min) was computed and used for analysis.

RESULTS

Mortality prior to DO trials. A large number of larvae perished before their use in the DO trials, resulting in a limited number of larvae available the DO trials and a non-random pattern to those mortalities. Deaths were complete for all individuals exposed to TCDD at doses > 0.001 ppm (NB sourced fish) or > 0.01 (SC fish), and for most of the higher doses of PCB126 with the exception of 0.1 ppm (NB fish) and 0.01, and 0.1 (SC fish). All remaining fish were used up to a maximum of 40 per toxicant exposure treatment (i.e., type and dose of toxicant), resulting in DO trials being conducted on 154 and 200 larvae for NB and SC-sourced fish, respectively.

DO regimes. The DO system used here for short-term, 21-hr duration trials was effective in producing the target DO levels (Figure 4). The most hypoxic treatment level (target 3-mg O_2/L) was the most difficult to maintain but the average over the 21-hr duration of the trial was 3.10 $\pm$ 0.014 mg O_2/L (Y$\pm$SEM). The 6-mg O_2/L nominal level varied most around the midpoint but the average was close to the nominal target value (6.08 $\pm$ 0.013).

Larval husbandry and size of fish at DO trials. The sizes of larvae used in the DO trials varied within and between populations (Figure 5). Sturgeon larvae from the NB population covered a wider range of sizes and were, on average, larger at the time of the trials (mean: 42.6; range: 10.9 to 80.1 mm SL) than the larvae from the SC source (mean: 27.2; range: 19.4 to 50.8 mm SL). The variation within populations was due to intrinsic differences in growth rates and the different ages of fish used in the trials. Any one trial used a maximum of 40 fish and trials were conducted between 10/08/2015 and 11/05/2015.

Response to the combined stressors of toxicants and dissolved oxygen.

Mortality during DO trials. Only one of 154 (< 1%) NB sourced larvae died during the DO trials

whereas 36 of 200 (36%) SC sourced larvae died during the trials. No significant main effects (toxicant, DO) or interactions were detected for the NB-sourced larvae. The mortality pattern among SC larvae was associated mostly with the Aroclor and TCDD, with DO also significant in the DO experiments (Table 2). No interaction term was deemed significant. Mortality during the earlier life-stages (prior to DO exposures), including embryo and early larval mortality, was significantly and negatively affected by all three toxicants (Chambers and Wirgin, in preparation).

Prey consumption during DO trials. Larvae were offered one of three types of prey during the trials – live *Artemia* and white worms (*Enchytraeus albidus*) or thawed frozen bloodworms (*Chironomus* sp.). NB-sourced larvae exhibited significant effects of DO but not in a general trend between prey consumption level and DO. In both cases, larvae in the intermediate DO level (6 mg O_2/L) exhibited the lowest consumption rate. The SC-sourced larvae exhibited sensitivities to both environmental stressors. Prey consumption was significantly affected by Aroclor and DO in the Aroclor-DO experiment, and by PCB126 and DO in that experiment (Table 2). Regarding the difference in responses between NB and SC-sourced larvae, the latter may be intrinsically more sensitive to these stressors and/or the experimental protocol was more likely to detect SC sensitivities due to the somewhat smaller sizes of SC larvae than NB larvae used in these trials (Figure. 5). Smaller larvae were likely to be less capable of consuming all prey offered during a trial than larger larvae, and 100% prey consumption was frequently observed in the trials thus obviating analyses of patterns of variances.

Activity of larvae during DO trials. Larval activity, scored as the average fish velocity during the video segments within a trial, did not show a significant effect for either stressor in either population. The lack of DO effects on activity is somewhat surprising because we expected behavior (i.e., movement) to be a labile character that is likely to be one of the first affected by stressors in the environment. In some cases, the trend with toxicant was towards more activity at higher doses. The pattern with respect to DO suggest a trend towards lower activity at lower DO levels. Possibly environmental stressors such as low DO may elicit a response in a finer time grain than the data we analyzed (our summary data were total distance traveled in two, 30-min video session, and the average velocity exhibited by larvae during this time). Samples sizes for activity were generally lower than those for the other two responses (mortality and prey consumption) so analyses of activity had less power to distinguish effects of these stressors. Suggestions for future studies would be to parse the timeline of behavior more finely into subsets during the video period, quantify patterns of stasis versus movement, and increase sample sizes.

SUMMARY AND FUTURE DIRECTIONS

We used exposure protocols to subject embryos of Atlantic sturgeon to a wide range of multiple dioxin-like compounds. We were able to maintain larvae from the less toxic compounds and lower toxicant concentrations up to 16 weeks post hatching and successfully use them in a newly developed DO delivery system.

The single and joint effects of DO and toxicants expressed themselves differently between the two tested populations. The SC-sourced Atlantic sturgeon larvae were more sensitive than NB larvae, especially with respect to mortality. Prey consumption appeared to be the most labile

response measured. Larval activity, at least as measured here, was relatively resilient to toxicants and DO.

Regarding our initial objectives, the challenge of Atlantic sturgeon larvae by hypoxia (Objective 1) showed DO to have a marginally significant (DO x TCDD experiment) to significant (DO x Aroclor experiment) effect on prey consumption rate but only for the SC-sourced larvae. We evaluated this by subjecting larvae to a wide range of DO values (10 to 3 mg/L) to mimic a short-term, acute hypoxia pinch. These results suggest that prey consumption by larval sturgeon of Atlantic sturgeon at low DO levels could be affected *in situ*. We recommend running experiments using a wider and younger age range of sturgeon and subjecting them to a more prolonged low-DO exposure (see below).

Regarding interactive effects between DO and toxicants (Objective 2), our experiments showed a marginally significant effect ($0.10 < p < 0.05$) interaction effect of low DO and the Aroclor mixtures that we employed. This effect was again only seen in prey consumption rate which underscores the value of this behavioral metric for scoring functional responses to environmental stressors.

For future directions, we suggest the following.

1. Develop behavioral responses with higher temporal frequency detection capabilities.
2. Initiate exposures earlier in larval life and perhaps during the embryonic period, and subject larvae to low DO for longer.
3. Develop higher throughput assays of response to DO to increase sample sizes overall and hence statistical power for resolving subtle differences.
4. Develop a DO system that provides diel variations in DO levels to mimic day-night differences in DO as occurs *in situ*.

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TABLE1. Experimental designs for testing effects of toxicants and dissolved oxygen (DO) on Atlantic sturgeon larvae. Although toxicant exposures of embryos were conducted at three concentrations of each toxicant, mortalities prior to the DO trials with deaths predominantly in the higher doses of TCDD and PCB126 resulted in unbalanced designs and low sample sizes for responses measured. For each group, responses measured during DO exposure were mortality, prey consumption, and activity level. Each toxicant treatment included a water and acetone (solvent) control. Abbreviations: NB (New Brunswick, Canada); SC (South Carolina).

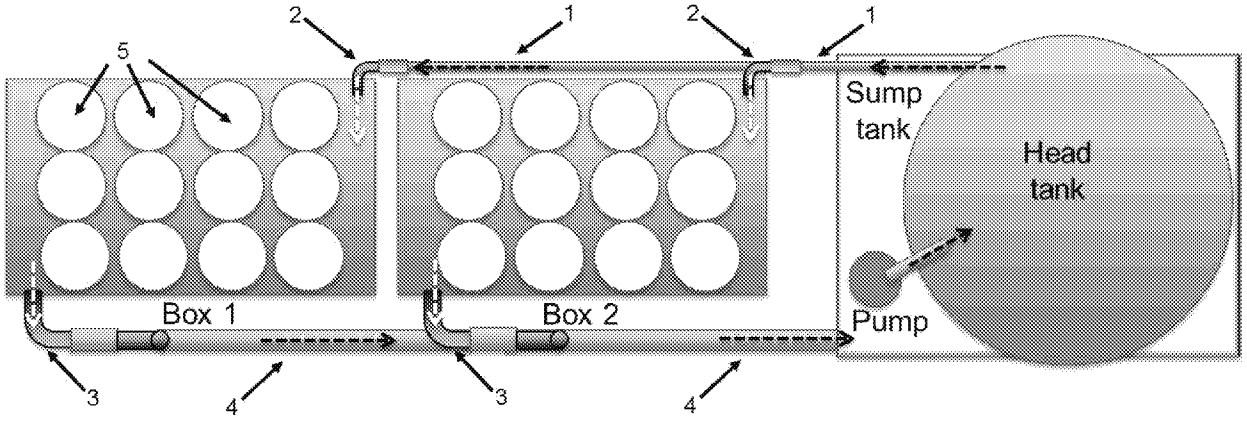
Source	Treatment Toxicant	Treatment dose (ppb)	Treatment Dissolved oxygen (mgO_2/L)	Replicates
NB	Aroclor mixture	0.2, 20, 2000	10, 8, 6, 4, 3	4
	PCB126	0.1	10, 8, 6, 4, 3	4
	TCDD	0.001	10, 8, 6, 4, 3	4
SC	Aroclor mixture	2, 20, 200	10, 8, 6, 4, 3	5
	PCB126	0.01, 0.1	10, 8, 6, 4, 3	5
	TCDD	0.01	10, 8, 6, 4, 3	5

Table 2. Summary of tests of effects of toxicants (Aroclor mixture, PCB126, and TCDD) and dissolved oxygen (DO) on Atlantic sturgeon larvae during short-term (21-hr) DO trials. Tests for no effect of treatments and interactions on outcomes were conducted by first applying a 2-way MANOVA with interaction (none was significant) followed by 2-way ANOVAs on each of three response variables (mortality, prey consumption, and activity). Details for significant ANOVAs are provided in subsequent Tables A1 to A18 (Table number referenced below). Abbreviations: Pop. (source population); SC (South Carolina); NB (New Brunswick, Canada); PCB (polychlorinated biphenyl congener 126); TCDD (2,3,7,8 tetrachlorodibenzo-*p*-dioxin); NS (not significant, $p > 0.05$). DO, mg O_2/L; toxicants, parts per billion (ppb).

Pop	Source term	Levels	Response variable		
			Mortality	Prey consumed	Activity
SC	Aroclor	2, 20, 200	p=0.010	p<0.001	NS
	DO	10, 8, 6, 4, 3	NS	p=0.011	NS
	Aro x DO		NS	NS	NS
		Table:	A1	A2	A3
		Figure:	A1	A2	A3
	PCB126	0.01, 0.1	NS	p=0.018	NS
	DO	10, 8, 6, 4, 3	NS	p=0.006	NS
	PCB x DO		NS	NS	NS
		Table:	A4	A5	A6
		Figure:	A4	A5	A6
	TCDD	0.01	p=0.030	NS	NS
	DO	10, 8, 6, 4, 3	p=0.040	NS	NS
	TCDD x DO		NS	NS	NS
		Table:	A7	A8	A9
		Figure:	A7	A8	A9
NB	Aroclor	0.2, 20, 2000	NS	NS	NS
	DO	10, 8, 6, 4, 3	NS	NS	NS
	Aro x DO		NS	NS	NS
		Table:	A10	A11	A12
		Figure:	A10	A11	A12
	PCB126	0.1	NS	NS	NS
	DO	10, 8, 6, 4, 3	NS	p<0.001	NS
	PCB x DO		NS	NS	NS
		Table:	A13	A14	A15
		Figure:	A13	A14	A15
	TCDD	0.001	NS	NS	NS
	DO	10, 8, 6, 4, 3	NS	p<0.001	NS
	TCDD x DO		NS	NS	NS
		Table:	A16	A17	A18
		Figure:	A16	A17	A18

Figure 1. A) Diagram of one of five dissolved oxygen (DO) delivery system for fish testing (plane view). Each system has a head tank which supplies treatment water to two rectangular boxes. Each box, plumbed with inflow (1, 2) and outflow (3, 4), has capacity to hold 12 test arenas (5). A submersible pump in the sump tank returns water to the head tank (after filtration). A DO probe in the head tank monitors DO levels, sends monitored DO values to a controller, and the controller adjusts N_2 gas flow to head tank via opening / closing of in-line solenoid valve to maintain DO levels at target values. B) Diagram of DO delivery system (lateral view). Water level set by outflow and air release adapter (6).

A)



B)

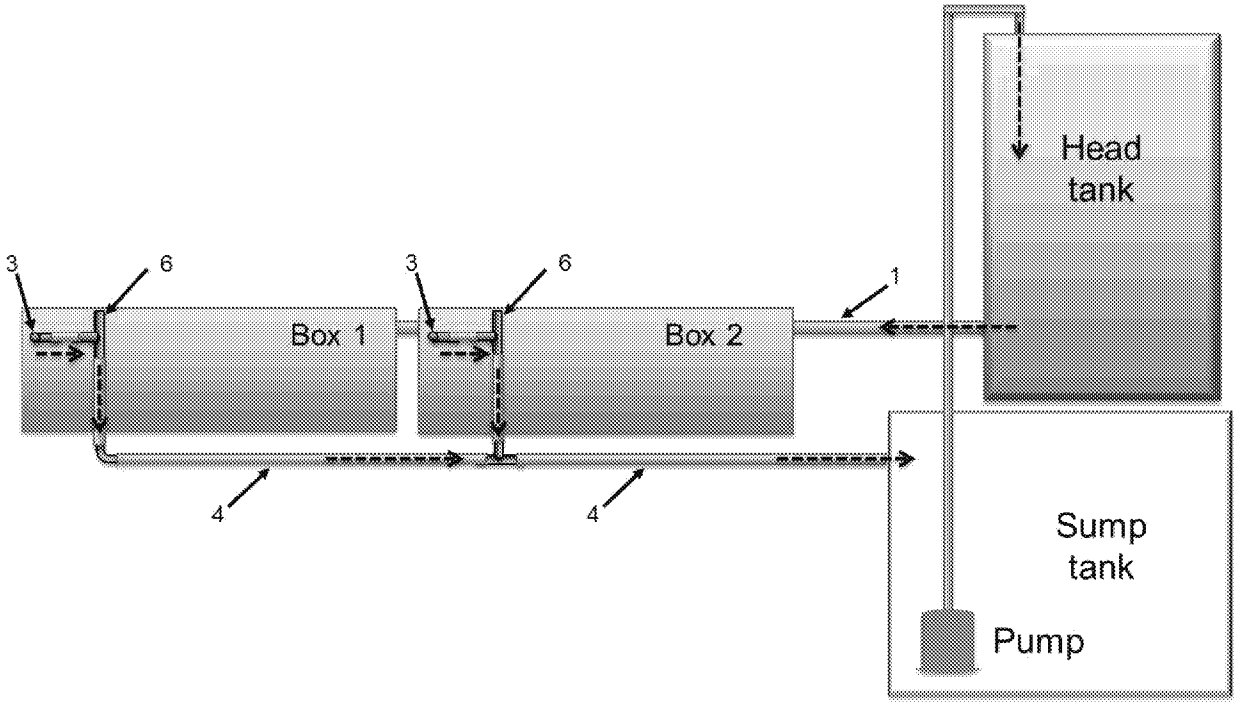


Figure 2. Photograph of dissolved oxygen (DO) delivery system for fish testing. Each cylindrical head tank supplies water of prescribed DO levels to a pair of associated rectangular boxes with each box containing up to 12 individual fish arenas. A single larva with prey was allocated to each arena. Larval mortality, prey consumption, and activity were scored during trial. Water departs each box, is collected in sump (not shown), and pumped through cartridge filtration system before returning to head tank. A DO probe in each head tank monitors DO levels, sends monitored DO values to a controller, and the controller adjusts N gas flow to the head tank via opening / closing of in-line solenoid valve to maintain DO levels at target values. During trials, each box is covered with a plane of tempered glass with gasket seal to minimize O_2 absorption from room air.

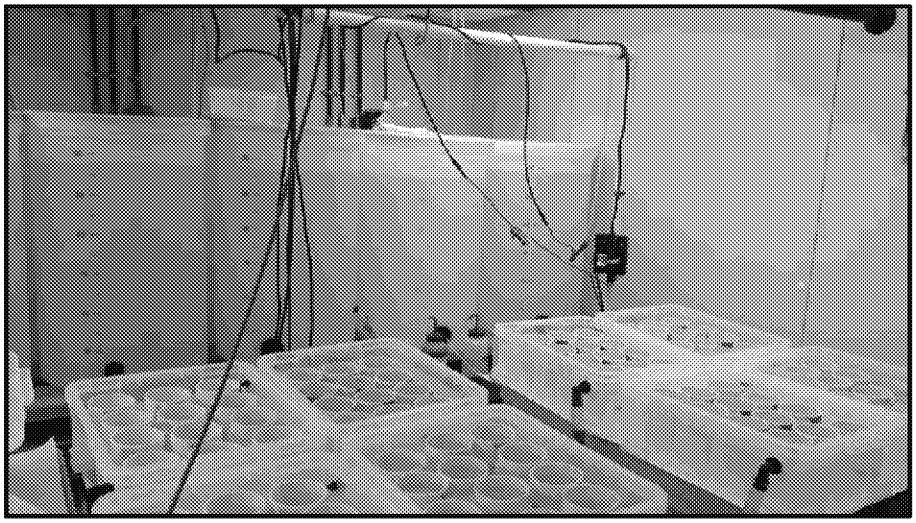


Figure 3. Look-down perspective of individual arenas in DO system for fish testing. In a trial, each arena contains an individual sturgeon larva (9 shown here). Larval mortality, prey consumption, and activity (e.g., swimming velocity) are scored during trial.

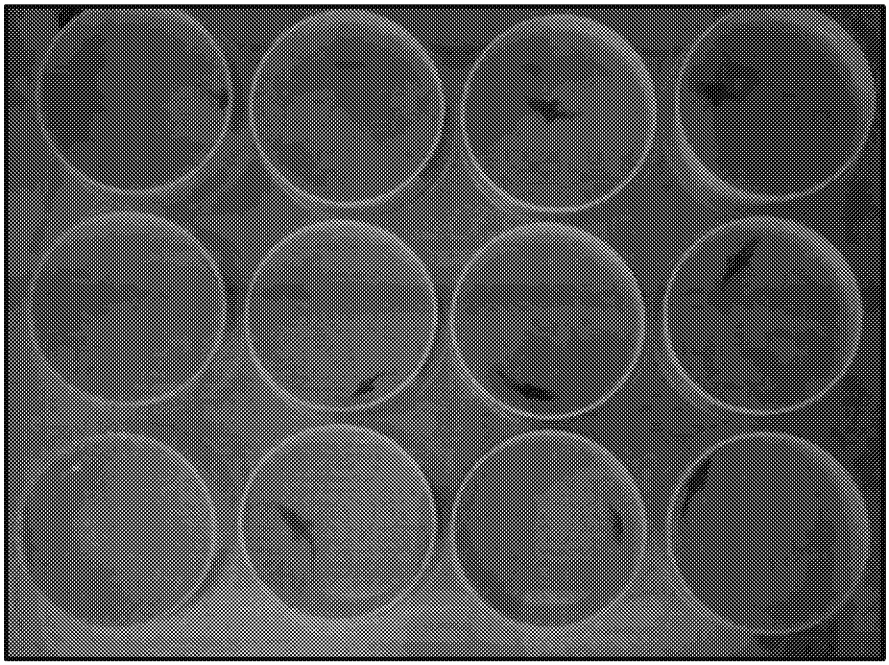


Figure 4. Dissolved oxygen (DO) levels used and the time course of Do levels during short-term exposure of Atlantic sturgeon larvae. Individual larvae were placed into arenas, acclimated, and at time T_0, N_2 gas was added to system head tank to purge O_2 to prescribed levels in exposure system. Rate of reduction of DO was approximately 1 mg O_2/L per hr. Once target DO were achieved (1 to 8 hr after addition of N gas), food was added and the 21-hr trial was initiated.

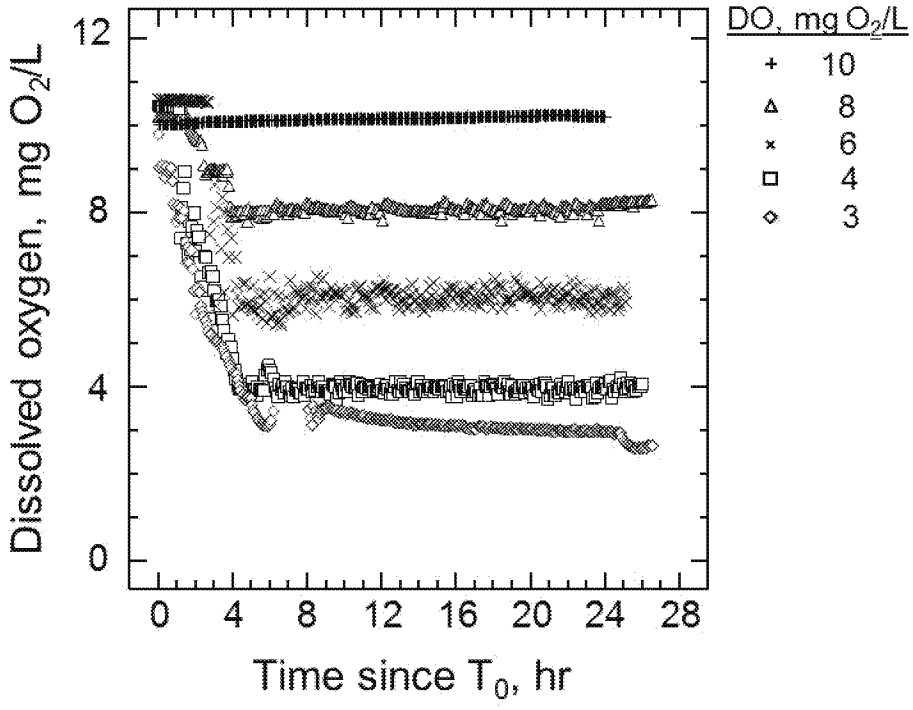
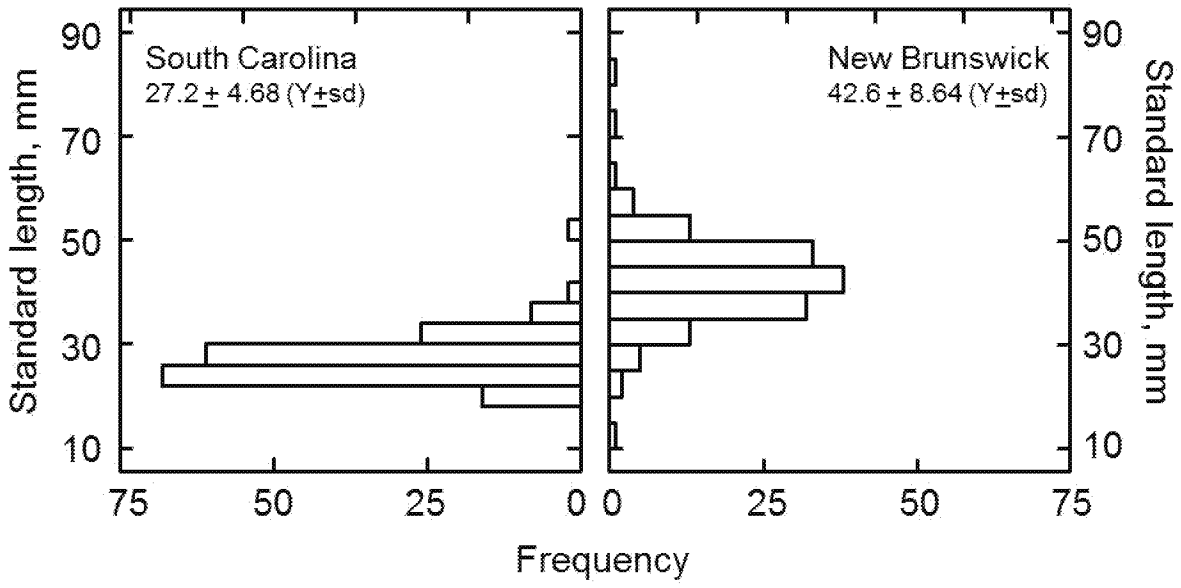


Figure 5. Size (mm SL) of Atlantic sturgeon larvae used in dissolved oxygen (DO) trials. Larvae from two sources, South Carolina (N= 200) and New Brunswick (N = 154), had been treated with one of three toxicants (Aroclor mixture, PCB126, or TCDD) as embryos and prior to DO exposure.



APPENDIX. 1.

TABLES. Collection of analysis of variance tables for each experiment (Aroclor x DO, PCB126 x DO, and TCDD x DO) for each of two populations (South Carolina and New Brunswick, Canada). Main effects are shown only if one or more terms are significant.

Table A1. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **mortality** of South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.22, N=124.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
Aroclor mixture	1.873	4	0.468	3.525	0.010
Dissolved Oxygen	0.659	4	0.165	1.241	0.299
Aroclor x DO	1.086	16	0.068	0.511	0.936
Error	13.150	99	0.133		

Table A2. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **prey eaten** by South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.35, N=117.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
Aroclor mixture	2.447	4	0.612	5.970	0.000
Dissolved Oxygen	1.415	4	0.354	3.453	0.011
Aroclor x DO	1.463	16	0.091	0.892	0.580
Error	9.426	92	0.102		

Table A3. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **activity of** South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.55, N=31.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
Model	48761	16	3048	1.052	0.467
Error	40570	14	2898		

Table A4. Tests of effects of PCB126 and dissolved oxygen (DO) exposure on **mortality** of South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.22, N=99.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
PCB126	0.865	3	0.288	2.290	0.085
Dissolved Oxygen	0.976	4	0.244	1.936	0.113
PCB126 x DO	0.931	12	0.078	0.616	0.823
Error	9.950	79	0.126		

Table A5. Tests of effects of PCB126 and dissolved oxygen (DO) exposure on **prey eaten** by South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.32, N=91.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
PCB126	1.234	3	0.411	3.560	0.018
Dissolved Oxygen	1.801	4	0.450	3.896	0.006
PCB126 x DO	1.040	12	0.087	0.750	0.698
Error	8.205	71	0.116		

Table A6. Tests of effects of PCB126 mixture and dissolved oxygen (DO) exposure on **activity of** South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.51, N=24.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
Model	36520	11	3320	1.122	0.421
Error	35499	12	2958		

Table A7. Tests of effects of TCDD and dissolved oxygen (DO) exposure on **mortality** of South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.22$, N=99.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
TCDD	1.059	2	0.530	3.741	0.030
Dissolved Oxygen	1.520	4	0.380	2.685	0.040
TCDD x DO	0.997	8	0.125	0.881	0.538
Error	8.350	59	0.142		

Table A8. Tests of effects of TCDD and dissolved oxygen (DO) exposure on **prey eaten** by South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.31$, N=65.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
Model	2.976	13	0.229	1.789	0.071
Error	6.527	51	0.128		

Table A9. Tests of effects of TCDD mixture and dissolved oxygen (DO) exposure on **activity of** South Carolina-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.61$, N=17.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
Model	33704	8	4213	1.558	0.272
Error	21631	8	2703		

Table A10. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **mortality** of New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.16, N=106.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
Aroclor mixture	0.017	4	0.004	0.402	0.807
Dissolved Oxygen	0.022	4	0.005	0.525	0.718
Aroclor x DO	0.095	16	0.006	0.580	0.891
Error	0.833	81	0.010		

Table A11. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **prey eaten** by New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.59, N=96.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
Model	7.494	21	0.357	5.059	0.000
Error	5.220	74	0.071		

Table A12. Tests of effects of Aroclor mixture and dissolved oxygen (DO) exposure on **activity of** New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.27, N=60.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
Model	22674	21	1080	0.673	0.832
Error	60921	38	1603		

Table A13. Tests of effects of PCB126 and dissolved oxygen (DO) exposure on **mortality** of New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.15$, N=68.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
PCB126	0.014	2	0.007	0.444	0.644
Dissolved Oxygen	0.042	4	0.011	0.669	0.616
PCB126 x DO	0.083	8	0.010	0.656	0.727
Error	0.833	53	0.016		

Table A14. Tests of effects of PCB126 and dissolved oxygen (DO) exposure on **prey eaten** by New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.42$, N=68.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
PCB126	0.137	2	0.069	0.781	0.463
Dissolved Oxygen	2.589	4	0.647	7.353	0.000
PCB126 x DO	0.675	8	0.084	0.958	0.478
Error	4.665	53	0.088		

Table A15. Tests of effects of PCB126 mixture and dissolved oxygen (DO) exposure on **activity of** New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model $R^2 = 0.51$, N=44.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
PCB126	4666	2	2333	1.454	0.250
Dissolved Oxygen	12906	4	3227	2.011	0.119
PCB126 x DO	24584	8	3073	1.915	0.096
Error	46527	29	1604		

Table A16. Tests of effects of TCDD and dissolved oxygen (DO) exposure on **mortality** of New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.15, N=68.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Mortality					
TCDD	0.014	2	0.007	0.444	0.644
Dissolved Oxygen	0.042	4	0.011	0.669	0.616
TCDD x DO	0.083	8	0.010	0.656	0.727
Error	0.833	55	0.016		

Table A17. Tests of effects of TCDD and dissolved oxygen (DO) exposure on **prey eaten** by New Brunswick-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.44, N=68.

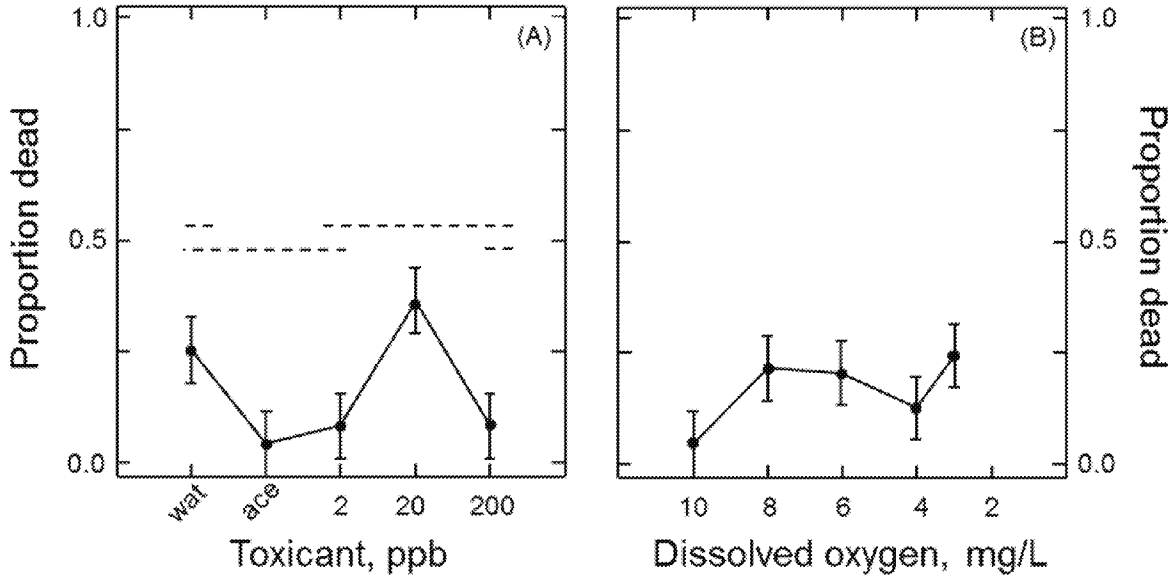
Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Proportion of prey eaten					
TCDD	0.043	2	0.021	0.258	0.773
Dissolved Oxygen	3.149	4	0.787	9.528	0.000
TCDD x DO	0.265	8	0.033	0.402	0.915
Error	4.380	53	0.083		

Table A18. Tests of effects of TCDD mixture and dissolved oxygen (DO) exposure on **activity of** New Brunswick [EDIT]-sourced Atlantic sturgeon larvae during short-term (21-hr) trials. Summary of results from 2-way ANOVA. Model R^2 = 0.37, N=44.

Treatment	Sum-of-squares	df	Mean square	F-ratio	p-value
Movement					
TCDD	5965	2	2983	1.820	0.180
Dissolved Oxygen	10487	4	2622	1.600	0.201
TCDD x DO	13099	8	1637	0.999	0.458
Error	47530	29	1639		

FIGURES. Collection of one-factor and two-factor plots for each experiment (Aroclor x DO, PCB126 x DO, and TCDD x DO) and populations (South Carolina and New Brunswick, Canada). Bivariate plots are only shown if a main effect was significant. Multiple range tests were applied if a main effect was significant and are shown if significant subsets of responses among treatment levels were identified based on the Bonferroni criterion.

Figure A1. Proportion of South Carolina-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of (A) Aroclor concentration when exposed as embryos, (B) DO concentration, and (C) Aroclor x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.



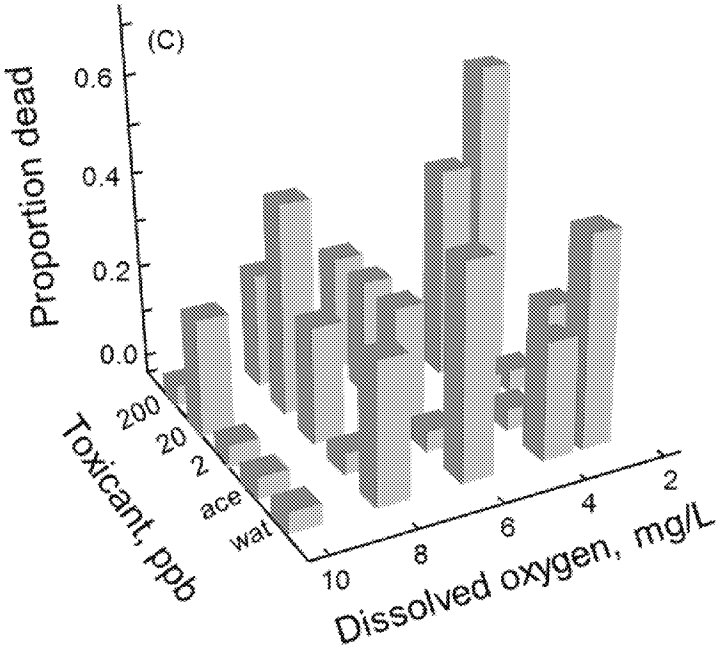
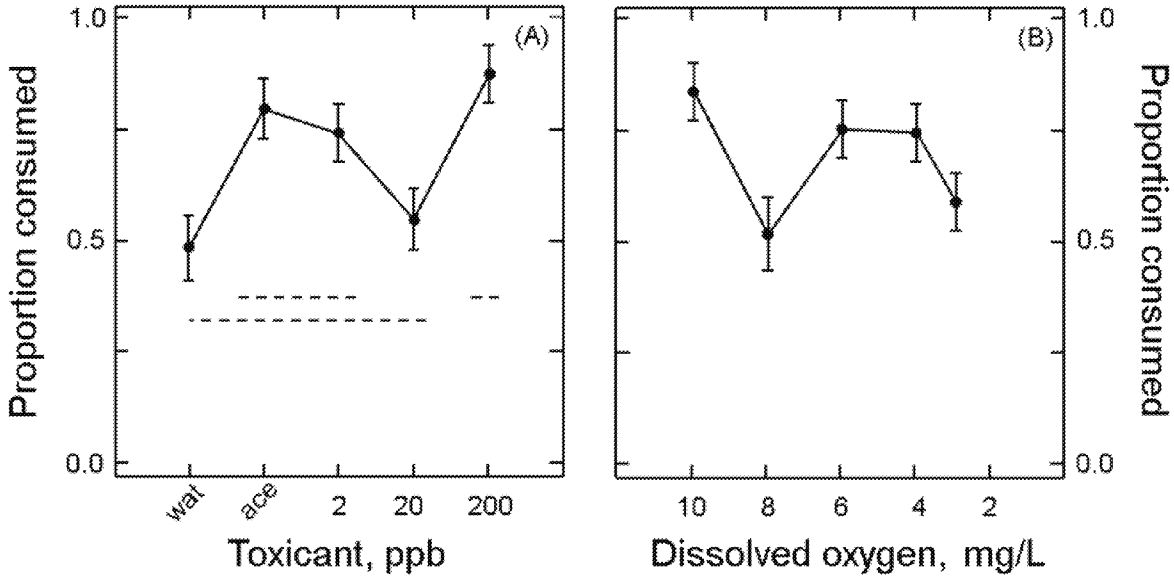


Figure A2. Proportion of prey consumed by South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of (A) Aroclor concentration when exposed as embryos, (B) DO concentration, and (C) Aroclor x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.



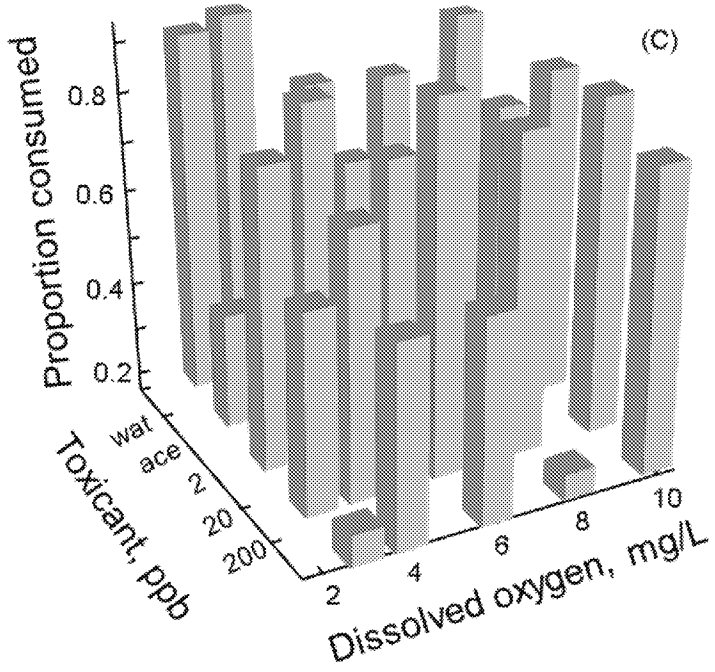


Figure A3. Movement of larval South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of Aroclor and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

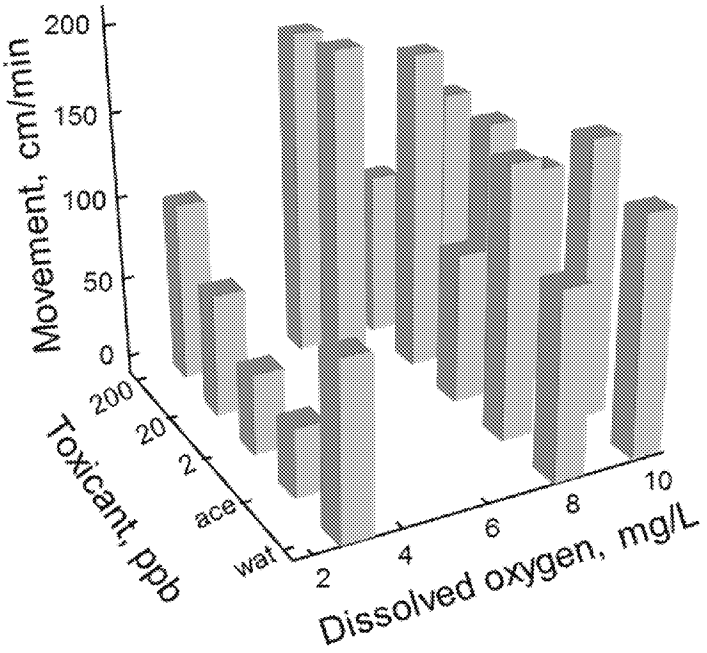


Figure A4. Proportion of South Carolina-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of PCB126 and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

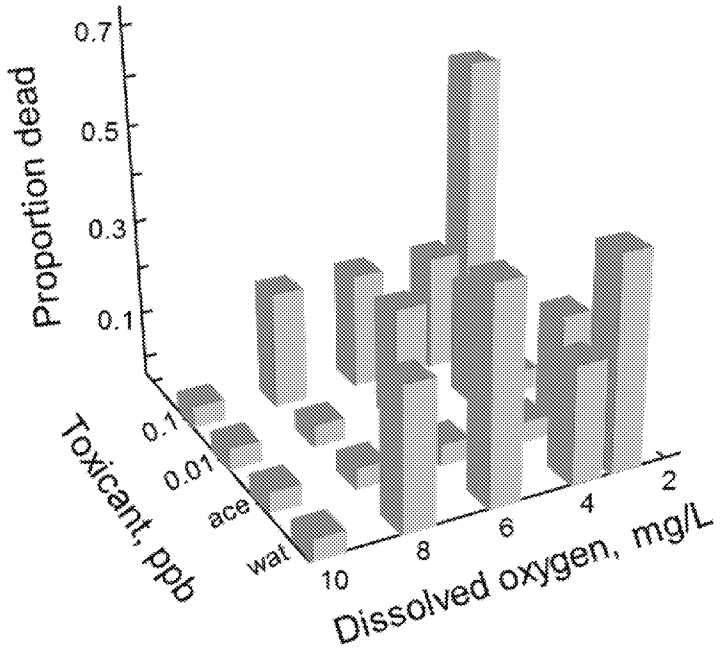


Figure A5. Proportion of prey consumed by South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of (A) PCB126 concentration when exposed as embryos, (B) DO concentration, and (C) PCB126 x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.

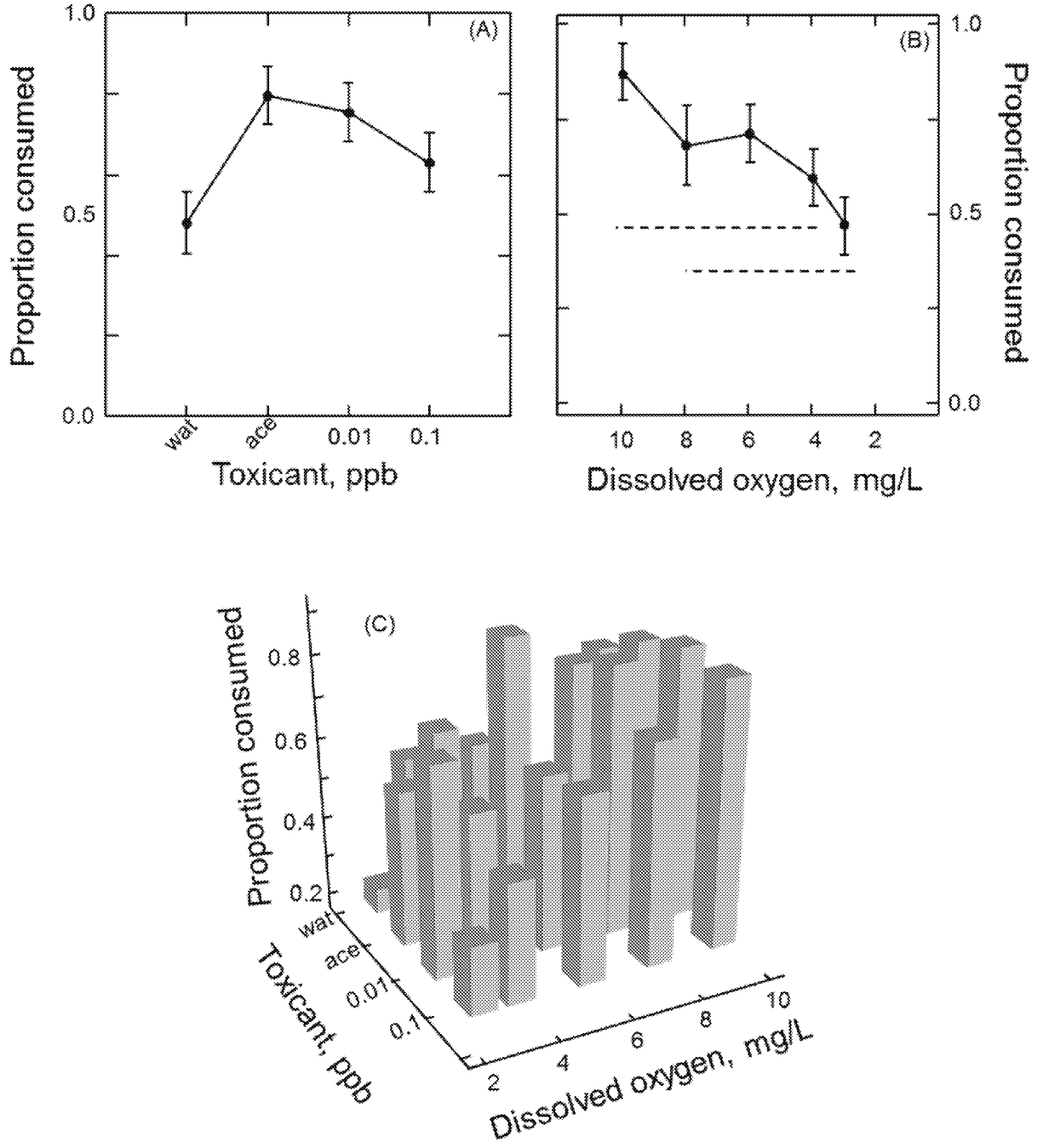


Figure A6. Movement of larval South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of PCB126 and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

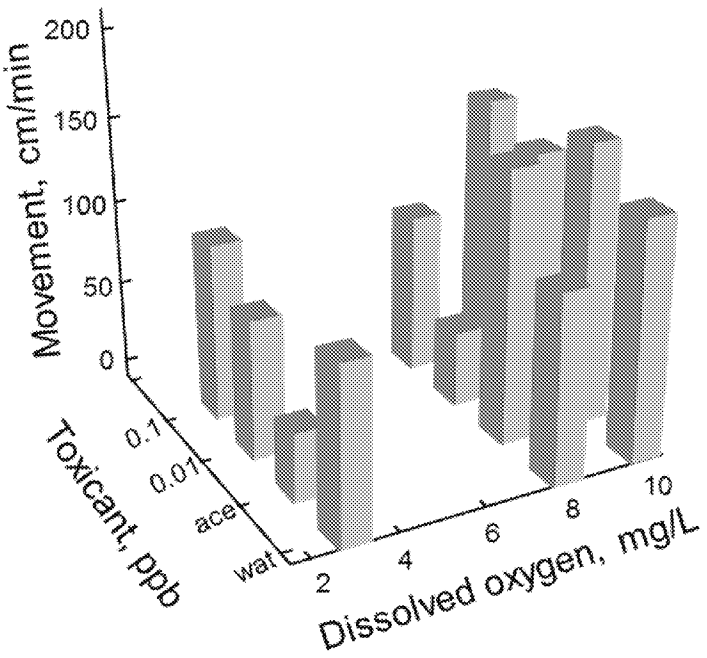
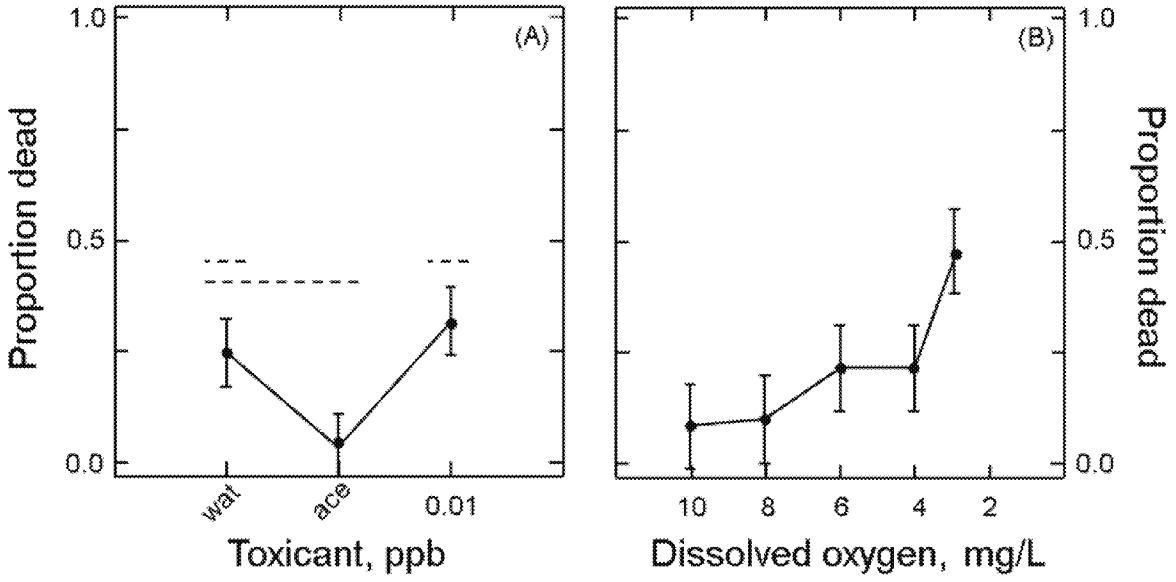


Figure A7. Proportion of South Carolina-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of (A) TCDD concentration when exposed as embryos, (B) DO concentration, and (C) TCDD x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.



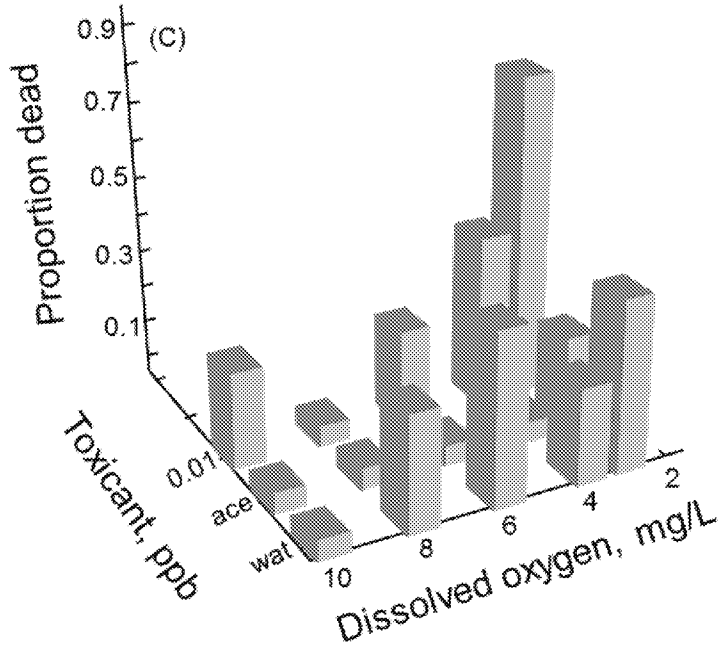


Figure A8. Proportion of prey consumed by South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of TCDD and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

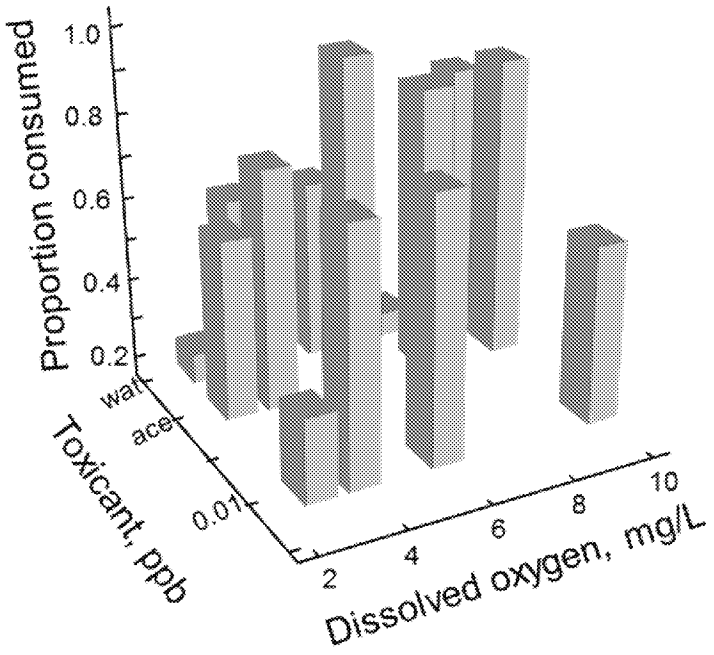


Figure A9. Movement of larval South Carolina-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of TCDD and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

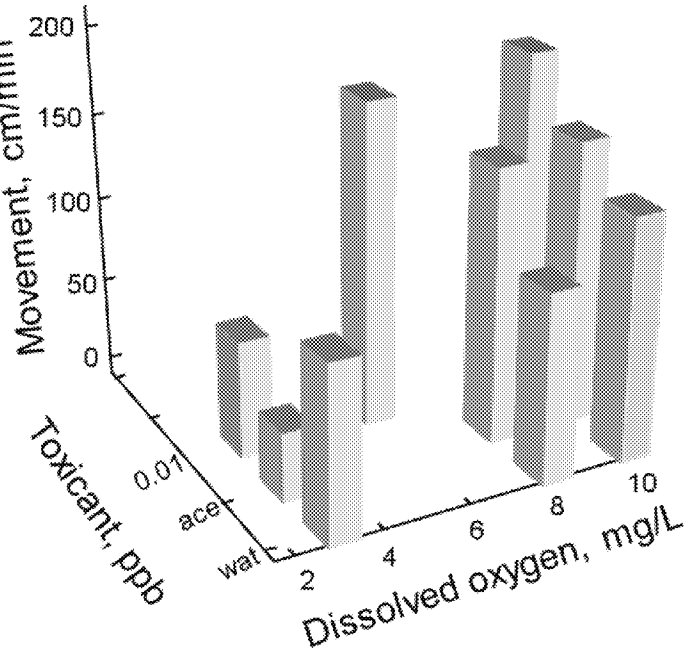


Figure A10. Proportion of New Brunswick-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of Aroclor and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

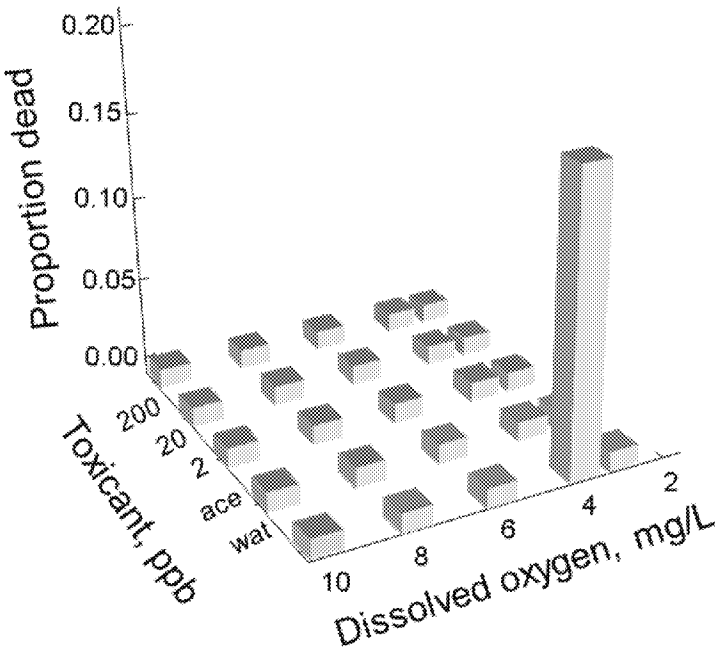


Figure A11. Proportion of prey consumed by New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of Aroclor and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

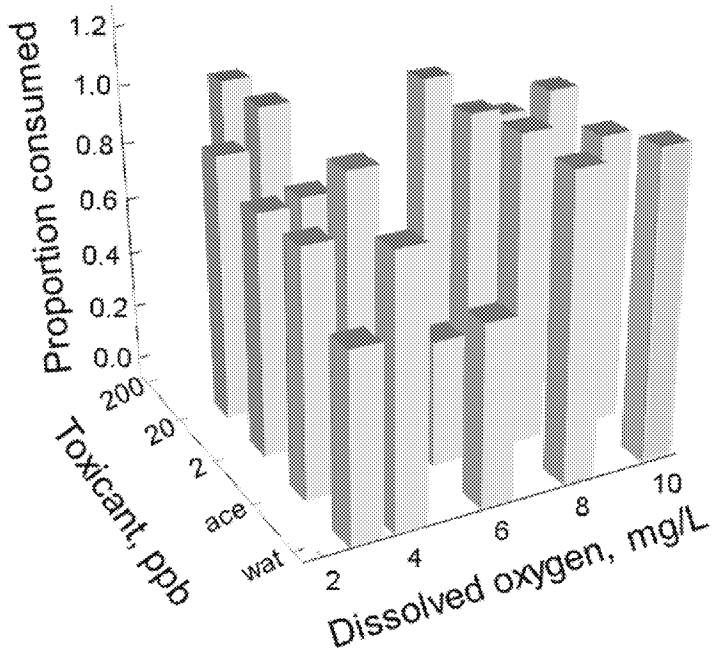


Figure A12. Movement of larval New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of Aroclor and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control. Abbreviations: wat = water control; ace = acetone control.

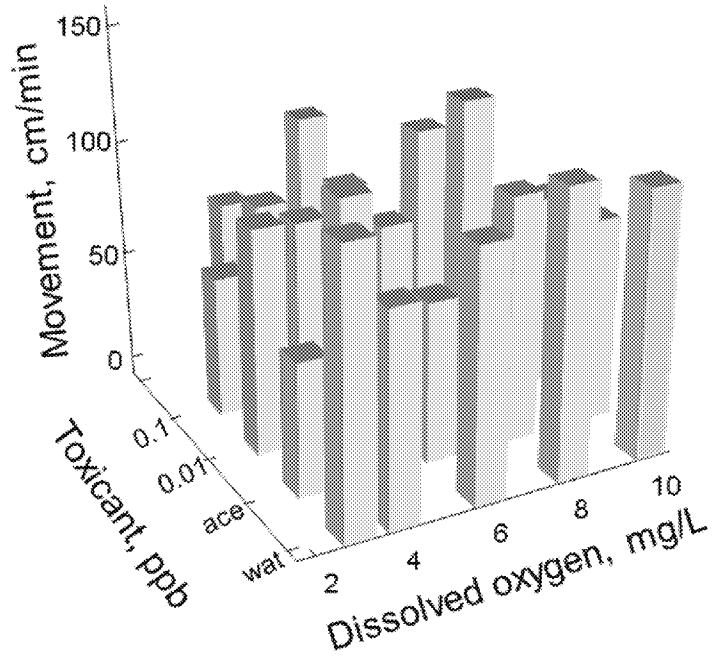


Figure A13. Proportion of New Brunswick-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of PCB126 and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

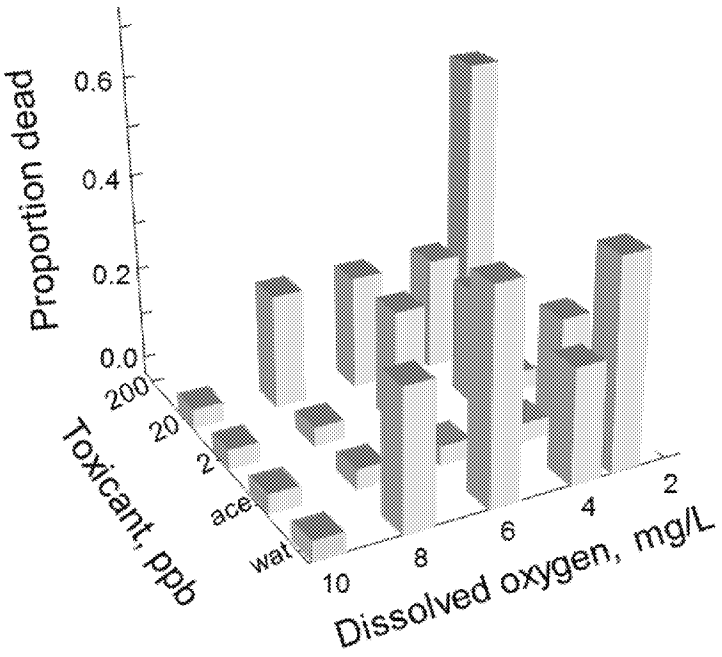
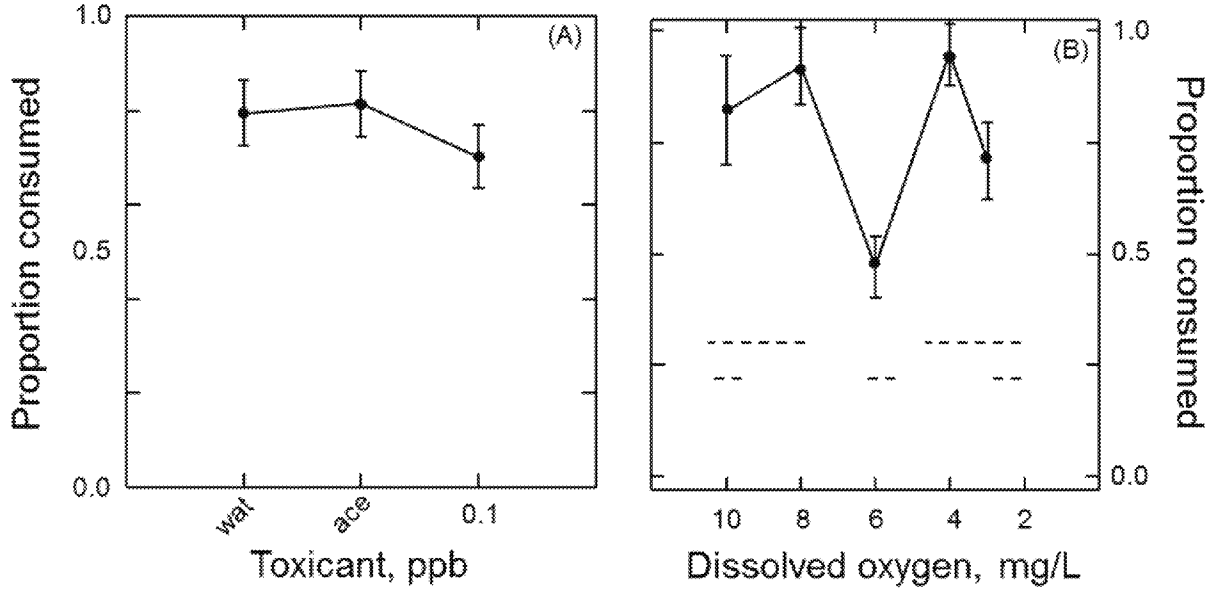


Figure A14. Proportion of prey consumed by New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of (A) PCB126 concentration when exposed as embryos, (B) DO concentration, and (C) PCB126 x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.



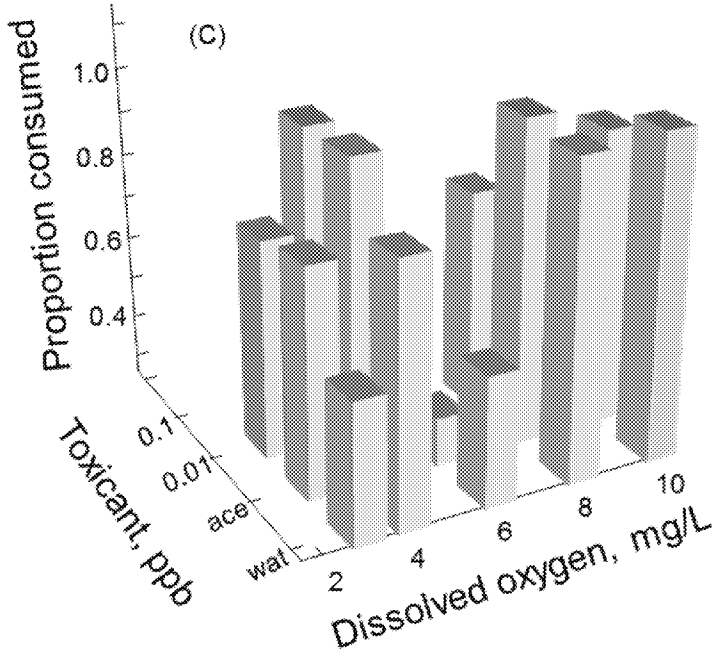


Figure A15. Movement of larval New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of PCB126 and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control. Abbreviations: wat = water control; ace = acetone control.

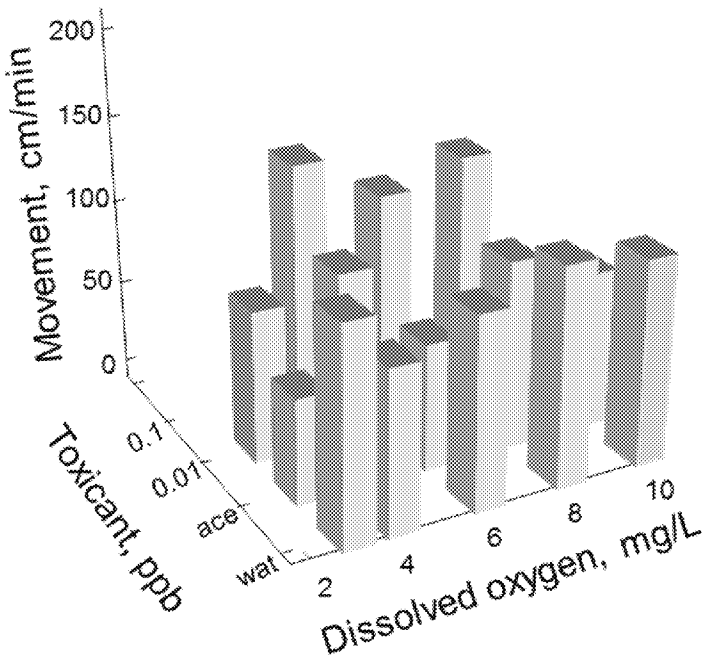


Figure A16. Proportion of New Brunswick-sourced Atlantic sturgeon dead after 21-hr dissolved oxygen (DO) trial as a function of TCDD and DO. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.

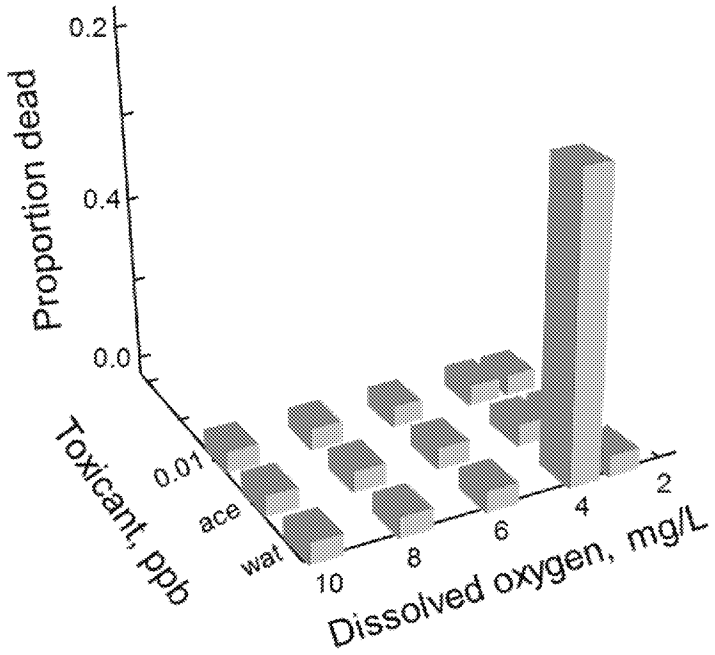
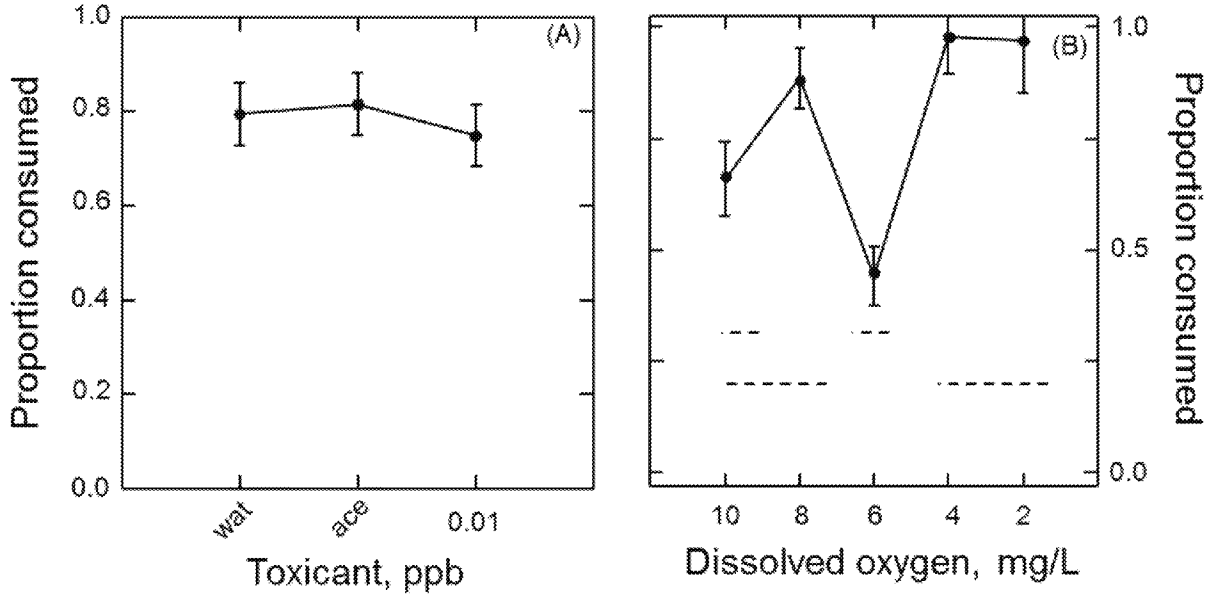


Figure A17. Proportion of prey consumed by New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of (A) TCDD concentration when exposed as embryos, (B) DO concentration, and (C) TCDD x DO interaction. Treatment level means plotted with (+/-) experiment-wide error in A, B. Bonferroni homogeneous subsets denoted by common elevations of dashed lines when significant. Abbreviations: wat = water control; ace = acetone control.



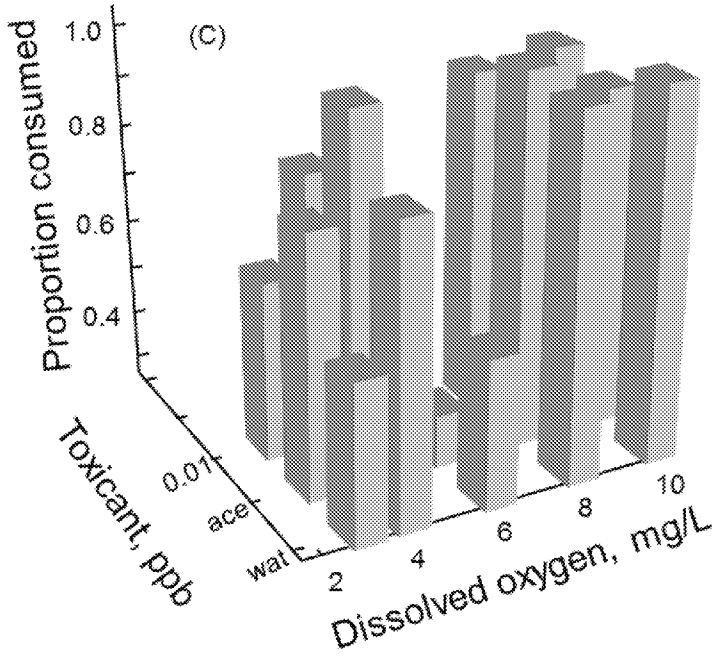


Figure A18. Movement of larval New Brunswick-sourced Atlantic sturgeon during 21-hr dissolved oxygen (DO) trial as a function of TCDD x DO interaction. Treatment level means plotted. Abbreviations: wat = water control; ace = acetone control.